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OIPe

## RAW SEQUENCE LISTING

PATENT APPLICATION: US/10/037,677

DATE: 02/07/2002

TIME: 19:15:31

Input Set : N:\Crf3\RULE60\10037677.txt

Output Set: N:\CRF3\02072002\J037677.raw

2 <110> APPLICANT: Schellenberger, Volker  
 3 Liu, Amy D.  
 4 Selifonova, Olga V.  
 6 <120> TITLE OF INVENTION: Directed Evolution of Microorganisms  
 8 <130> FILE REFERENCE: GC560  
 10 <140> CURRENT APPLICATION NUMBER: 10/037,677  
 11 <141> CURRENT FILING DATE: 2001-10-23  
 13 <150> PRIOR APPLICATION NUMBER: 09/314,847  
 14 <151> PRIOR FILING DATE: 1999-05-19  
 16 <160> NUMBER OF SEQ ID NOS: 15  
 18 <170> SOFTWARE: FastSEQ for Windows Version 3.0  
 20 <210> SEQ ID NO: 1  
 21 <211> LENGTH: 741  
 22 <212> TYPE: DNA  
 23 <213> ORGANISM: E. coli  
 25 <400> SEQUENCE: 1

26	atgaccgcta	tgagcactgc	aattacacgc	cagatcggtc	tcgataccga	aaccaccggt	60
27	atgaaccaga	ttggtgcgca	ctatgaaggc	cacaagatca	ttgagattgg	tgccggtgaa	120
28	gtggtgaacc	gtcgcttgac	gggcaataac	ttccatgttt	atctcaaacc	cgatcggtcg	180
29	gtggatccgg	aagccttttg	cgtacatggt	attgccgatg	aattttttgct	cgataagccc	240
30	acgtttgccc	aagtagccga	tgagttcatg	gactatatct	gcggcgcgga	gttggatgat	300
31	cataacgcag	cgttcgatat	cggcttttat	gactacgagt	tttcggttgc	taagcgcgat	360
32	attccgaaga	ccaatacttt	ctgtaaggtc	accgatagcc	ttgcggtggc	gaggaaaatg	420
33	tttcccggta	agcgcaacag	cctcgatgcg	ttatgtgctc	gctacgaaat	agataacagt	480
34	aaacgaacgc	tgacgggggc	attactcgat	gcccgatccc	ttgcggaagt	ttatctggcg	540
35	atgaccgggtg	gtcaaacgtc	gatggctttt	gcgatggaag	gagagacaca	acagcaacaa	600
36	ggtgaagcaa	caattcagcg	cattgtacgt	caggcaagta	agttacgcgt	tgtttttgcg	660
37	acagatgaag	agattgcagc	tcatgaagcc	cgtctcgatc	tggtgcagaa	gaaaggcgga	720
38	agttgcctct	ggcgagcata	a				741

40 <210> SEQ ID NO: 2  
 41 <211> LENGTH: 246  
 42 <212> TYPE: PRT  
 43 <213> ORGANISM: E. coli  
 45 <400> SEQUENCE: 2

46	Met	Thr	Ala	Met	Ser	Thr	Ala	Ile	Thr	Arg	Gln	Ile	Val	Leu	Asp	Thr
47	1			5					10				15			
48	Glu	Thr	Thr	Gly	Met	Asn	Gln	Ile	Gly	Ala	His	Tyr	Glu	Gly	His	Lys
49			20					25				30				
50	Ile	Ile	Glu	Ile	Gly	Ala	Val	Glu	Val	Val	Asn	Arg	Arg	Leu	Thr	Gly
51		35					40				45					
52	Asn	Asn	Phe	His	Val	Tyr	Leu	Lys	Pro	Asp	Arg	Leu	Val	Asp	Pro	Glu
53		50				55			60							
54	Ala	Phe	Gly	Val	His	Gly	Ile	Ala	Asp	Glu	Phe	Leu	Leu	Asp	Lys	Pro

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55 65              70              75              80
56 Thr Phe Ala Glu Val Ala Asp Glu Phe Met Asp Tyr Ile Arg Gly Ala
57              85              90              95
58 Glu Leu Val Ile His Asn Ala Ala Phe Asp Ile Gly Phe Met Asp Tyr
59              100              105              110
60 Glu Phe Ser Leu Leu Lys Arg Asp Ile Pro Lys Thr Asn Thr Phe Cys
61              115              120              125
62 Lys Val Thr Asp Ser Leu Ala Val Ala Arg Lys Met Phe Pro Gly Lys
63              130              135              140
64 Arg Asn Ser Leu Asp Ala Leu Cys Ala Arg Tyr Glu Ile Asp Asn Ser
65              145              150              155              160
66 Lys Arg Thr Leu His Gly Ala Leu Leu Asp Ala Gln Ile Leu Ala Glu
67              165              170              175
68 Val Tyr Leu Ala Met Thr Gly Gly Gln Thr Ser Met Ala Phe Ala Met
69              180              185              190
70 Glu Gly Glu Thr Gln Gln Gln Gln Gly Glu Ala Thr Ile Gln Arg Ile
71              195              200              205
72 Val Arg Gln Ala Ser Lys Leu Arg Val Val Phe Ala Thr Asp Glu Glu
73              210              215              220
74 Ile Ala Ala His Glu Ala Arg Leu Asp Leu Val Gln Lys Lys Gly Gly
75              225              230              235              240
76 Ser Cys Leu Trp Arg Ala
77              245
79 <210> SEQ ID NO: 3
80 <211> LENGTH: 1164
81 <212> TYPE: DNA
82 <213> ORGANISM: E. blatte
84 <400> SEQUENCE: 3
85 atgagctatc gtatgtttga ttatctgtggt ccaaagtga acttcttttg cccgggcgcc 60
86 gtttctgttg ttggccagcg ctgccagctg ctggggggtg aaaaagccct gctggtgacc 120
87 gataagggcc tgcgcgccat taaagacggt gctgtcgatc agaccgtgaa gcacctgaaa 180
88 gccgccggtg ttgaggtggt cattttogac ggggtcgagc cgaaccgaa agacaccaac 240
89 gtgctcgacg gcctggccat gtccgtaaa gagcagtgcg acatgataat caccgtcggc 300
90 ggcggcagcc cgcacgactg cggtaaaggc attggtattg cggccacca cccgggtgat 360
91 ctgtacagct atgccggtat cgaaacactc accaaccgcg tgccgcccac tattgcggtc 420
92 aacaccaccg ccgggaccgc cagcgaagtc acccgccact gcgtgctgac taacaccaa 480
93 accaaagtaa aatttgtgat tgtcagctgg cgcaacctgc cttcgtctc cattaacgat 540
94 ccgctgctga tgatcggaac gcccgccggg ctgaccgccc ccaccggtat ggatgccctg 600
95 acccagcgcg tagaggccta tatctccaaa gacgccaacc cggttaccga tgccctctgt 660
96 attcaggcca tcaaactgat tgccaccaac ttgcgccagg ccgtcgccct ggggaccaac 720
97 ctcaaagccc gtgaaaacat ggccctgcgc tctctgctgg ccgggatggc ctttaacaac 780
98 gccaacctgg gctatgttca cgccatggct caccagctgg gcggcctgta cgacatggcc 840
99 caccgggtgg cgaacgcggt cctgctgccc catgtctgcc gctataacct gattgccaac 900
100 ccggaataat ttgccgatc cgccaccttt atgggggaaa acaccaccgg tctttccacc 960
101 atggacgcag cggagctggc catcagcgcc attgccgctc tgtctaaaga tgtcgggata 1020
102 ccgcagcacc tgcgtgaact gggggtaaaa gagccgact tcccgtacat ggcagaaatg 1080
103 gccctgaaag acggcaacgc cttctctaac ccgcgcaaag ggaacgaaaa agagattgcc 1140
104 gacattttcc gccaggcatt ctga
106 <210> SEQ ID NO: 4

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107 <211> LENGTH: 387
108 <212> TYPE: PRT
109 <213> ORGANISM: E. blatte
111 <400> SEQUENCE: 4
112 Met Ser Tyr Arg Met Phe Asp Tyr Leu Val Pro Asn Val Asn Phe Phe
113   1           5           10           15
114 Gly Pro Gly Ala Val Ser Val Val Gly Gln Arg Cys Gln Leu Leu Gly
115           20           25           30
116 Gly Lys Lys Ala Leu Leu Val Thr Asp Lys Gly Leu Arg Ala Ile Lys
117           35           40           45
118 Asp Gly Ala Val Asp Gln Thr Val Lys His Leu Lys Ala Ala Gly Ile
119           50           55           60
120 Glu Val Val Ile Phe Asp Gly Val Glu Pro Asn Pro Lys Asp Thr Asn
121           65           70           75           80
122 Val Leu Asp Gly Leu Ala Met Phe Arg Lys Glu Gln Cys Asp Met Ile
123           85           90           95
124 Ile Thr Val Gly Gly Gly Ser Pro His Asp Cys Gly Lys Gly Ile Gly
125           100          105          110
126 Ile Ala Ala Thr His Pro Gly Asp Leu Tyr Ser Tyr Ala Gly Ile Glu
127           115          120          125
128 Thr Leu Thr Asn Pro Leu Pro Pro Ile Ile Ala Val Asn Thr Thr Ala
129           130          135          140
130 Gly Thr Ala Ser Glu Val Thr Arg His Cys Val Leu Thr Asn Thr Lys
131           145          150          155          160
132 Thr Lys Val Lys Phe Val Ile Val Ser Trp Arg Asn Leu Pro Ser Val
133           165          170          175
134 Ser Ile Asn Asp Pro Leu Leu Met Ile Gly Lys Pro Ala Gly Leu Thr
135           180          185          190
136 Ala Ala Thr Gly Met Asp Ala Leu Thr His Ala Val Glu Ala Tyr Ile
137           195          200          205
138 Ser Lys Asp Ala Asn Pro Val Thr Asp Ala Ser Ala Ile Gln Ala Ile
139           210          215          220
140 Lys Leu Ile Ala Thr Asn Leu Arg Gln Ala Val Ala Leu Gly Thr Asn
141           225          230          235          240
142 Leu Lys Ala Arg Glu Asn Met Ala Cys Ala Ser Leu Leu Ala Gly Met
143           245          250          255
144 Ala Phe Asn Asn Ala Asn Leu Gly Tyr Val His Ala Met Ala His Gln
145           260          265          270
146 Leu Gly Gly Leu Tyr Asp Met Ala His Gly Val Ala Asn Ala Val Leu
147           275          280          285
148 Leu Pro His Val Cys Arg Tyr Asn Leu Ile Ala Asn Pro Glu Lys Phe
149           290          295          300
150 Ala Asp Ile Ala Thr Phe Met Gly Glu Asn Thr Thr Gly Leu Ser Thr
151           305          310          315          320
152 Met Asp Ala Ala Glu Leu Ala Ile Ser Ala Ile Ala Arg Leu Ser Lys
153           325          330          335
154 Asp Val Gly Ile Pro Gln His Leu Arg Glu Leu Gly Val Lys Glu Ala
155           340          345          350
156 Asp Phe Pro Tyr Met Ala Glu Met Ala Leu Lys Asp Gly Asn Ala Phe

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```

157          355          360          365
158 Ser Asn Pro Arg Lys Gly Asn Glu Lys Glu Ile Ala Asp Ile Phe Arg
159          370          375          380
160 Gln Ala Phe
161 385
163 <210> SEQ ID NO: 5
164 <211> LENGTH: 12
165 <212> TYPE: DNA
166 <213> ORGANISM: Artificial Sequence
168 <220> FEATURE:
169 <223> OTHER INFORMATION: wild type mutD gene
171 <400> SEQUENCE: 5
172 atgaccgcta tg
174 <210> SEQ ID NO: 6
175 <211> LENGTH: 11
176 <212> TYPE: DNA
177 <213> ORGANISM: Artificial Sequence
179 <220> FEATURE:
180 <223> OTHER INFORMATION: pOS100 mutD mutated gene
182 <400> SEQUENCE: 6
183 ttgacgcttt g
185 <210> SEQ ID NO: 7
186 <211> LENGTH: 12
187 <212> TYPE: DNA
188 <213> ORGANISM: Artificial Sequence
190 <220> FEATURE:
191 <223> OTHER INFORMATION: pOS101 mutD mutated gene
193 <400> SEQUENCE: 7
194 gtgaccgctg tg
196 <210> SEQ ID NO: 8
197 <211> LENGTH: 11
198 <212> TYPE: DNA
199 <213> ORGANISM: Artificial Sequence
201 <220> FEATURE:
202 <223> OTHER INFORMATION: pOS102 mutD mutated gene
204 <400> SEQUENCE: 8
205 gtgccgctgt g
207 <210> SEQ ID NO: 9
208 <211> LENGTH: 12
209 <212> TYPE: DNA
210 <213> ORGANISM: Artificial Sequence
212 <220> FEATURE:
213 <223> OTHER INFORMATION: pOS104 mutD mutated gene
215 <400> SEQUENCE: 9
216 ttgaccgctt tg
218 <210> SEQ ID NO: 10
219 <211> LENGTH: 55
220 <212> TYPE: DNA
221 <213> ORGANISM: Artificial Sequence

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223 <220> FEATURE:  
 224 <223> OTHER INFORMATION: pos105 mutD mutated gene  
 226 <400> SEQUENCE: 10  
 227 gtgaccgctg tgagcacttg caattacacg ccagatcggtt ctcgataccg aaatc 55  
 229 <210> SEQ ID NO: 11  
 230 <211> LENGTH: 11  
 231 <212> TYPE: DNA  
 232 <213> ORGANISM: Artificial Sequence  
 234 <220> FEATURE:  
 235 <223> OTHER INFORMATION: pos106 mutD mutated gene  
 237 <400> SEQUENCE: 11  
 238 gtgaccgctt g 11  
 240 <210> SEQ ID NO: 12  
 241 <211> LENGTH: 28  
 242 <212> TYPE: DNA  
 243 <213> ORGANISM: Artificial Sequence  
 245 <220> FEATURE:  
 246 <223> OTHER INFORMATION: primer  
 248 <400> SEQUENCE: 12  
 249 cgcctccagc gcgacaatag cggccatc 28  
 251 <210> SEQ ID NO: 13  
 252 <211> LENGTH: 27  
 253 <212> TYPE: DNA  
 254 <213> ORGANISM: Artificial Sequence  
 256 <220> FEATURE:  
 257 <223> OTHER INFORMATION: primer  
 259 <400> SEQUENCE: 13  
 260 ccgactgaac taccgctccg cgttggtg 27  
 262 <210> SEQ ID NO: 14  
 263 <211> LENGTH: 36  
 264 <212> TYPE: DNA  
 265 <213> ORGANISM: Artificial Sequence  
 267 <220> FEATURE:  
 268 <223> OTHER INFORMATION: primer  
 270 <400> SEQUENCE: 14  
 271 tctgatacgg gatcctcaga atgcctggcg gaaaat 36  
 273 <210> SEQ ID NO: 15  
 274 <211> LENGTH: 42  
 275 <212> TYPE: DNA  
 276 <213> ORGANISM: Artificial Sequence  
 278 <220> FEATURE:  
 279 <223> OTHER INFORMATION: primer  
 281 <400> SEQUENCE: 15  
 282 gcgcgtcta gaattatgag ctatcgtatg tttgattatc tg 42

VERIFICATION SUMMARY

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